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Enantioselective Synthesis of Pactamycin, a Complex Antitumor Antibiotic

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Abstract

Medicinal application of many complex natural products is precluded by the impracticality of their chemical synthesis. Pactamycin, the most structurally-intricate aminocyclopentitol antibiotic, displays potent anti-prolific properties across multiple phylogenetic domains, but is highly cytotoxic. A limited number of analogs produced by genetic engineering technologies show reduced cytotoxicity against mammalian cells, renewing promise for therapeutic applications. For decades, an efficient synthesis of pactamycin amenable to analog derivatizations has eluded researchers. Herein, we present a short asymmetric total synthesis of pactamycin. An enantioselective Mannich reaction/symmetry-breaking reduction sequence was designed to enable assembly of the entire carbon core skeleton in under five steps and control critical three-dimensional (stereochemical) functional group relationships. This modular route totals fifteen steps and is immediately amenable for structural analog synthesis.

Complex organic molecules produced by bacteria have been relied upon for the treatment of numerous disease types for nearly a century (1–4); however, many naturally-derived compounds that exhibit interesting bioactivities are practically inaccessible via synthetic organic chemistry. A natural product's structural complexity can create an insurmountable impediment to the preparation of analogs that might exhibit improved characteristics. An ongoing challenge in the field of synthetic chemistry is the development of methods that close the gap between the efficiency of biosynthetic machinery and laboratory synthesis. Because of the inherent flexibility of the latter, success in this endeavor could provide access to useful structural variants that might otherwise be inaccessible.

Pactamycin (**1**, Fig. 1) was isolated from *Streptomyces pactum* var *pactum* in 1961 by researchers at the Upjohn Company (5). The bioactivity profile of this natural product is remarkable as it displays antitumor, antimicrobial, antiviral, and antiprotozoal properties by acting as a universal inhibitor of translocation (6–9). Within the ribosomal subunit in which it interacts, pactamycin mimics an RNA dinucleotide through interactions of its aniline and salicylate moieties with stem loops in the 16S RNA (10). Unfortunately, therapeutic benefits have yet to be realized due to high cytotoxicity (IC_{50} 95 nM against human diploid embryonic cell line MRC-5) (11). Pactamycin is a prototypical example of a promising bioactive natural product whose complexity hampers investigation of structure/activity relationships (SAR) that might lead to a serviceable therapeutic application and/or better understanding of intrinsic bioactivity.

Genetic engineering studies have reignited promise for medicinal application as 7-deoxy- and 8″-hydroxy-derivatives were isolated and displayed diminished cytotoxity (11–14). In the context of the work described herein, it is worth noting that Lu et al. contend that the

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structural complexity of **1** renders these and related structural modifications “inaccessible by synthetic organic chemistry” (12). Conversely, we have proceeded from the hypothesis that the genetic engineering approach to pactamycin analogs might be inherently limited by the biosynthetic machinery (15). While pactamycin is commercially available, a chemical approach to its synthesis could in principle provide far greater opportunity and flexibility for discovering and advancing useful compounds; however, this tactic will only be feasible in the presence of an efficient synthesis platform that rapidly develops the level of structural complexity that is present. In fact, synthetic interest in pactamycin has recently flourished, culminating in the landmark 32-step total synthesis from Hanessian and coworkers (16, 17), as well as numerous partial synthetic studies (18–22). Despite these creative, state-of-the-art approaches, a compelling case can be made that a more practical synthesis solution is needed.

In this report, we disclose a fifteen-step total synthesis of pactamycin which can immediately produce the natural product on milligram scale and a key branch point intermediate on gram scale. Emphasis was placed on both modular construction and introduction of functionality in its final desired form, enabling an approach amenable to derivatization for analog synthesis. Late-stage introduction of the aniline and salicylate binding elements provides an opportunity for future SAR studies.

Critical to our synthetic plan was the recognition of a hidden symmetry in the northeast quadrant of pactamycin (**1**). Depicted in Fig. 2A, the carbon chain connecting C4 and C8 can be extracted to a symmetrical α-ureido-2,4-pentanedione **2**. We envisaged simplified formation of the fully-substituted C1 center via a Mannich reaction. Due to the symmetrical methyl ketone substituents at C1, diastereoselectivity considerations are obviated, allowing for a focus on the enantioselective C2-amino incorporation during the C1–C2 bond construction. The nascent C2 stereocenter would then need to direct a site- and diastereoselective diketone mono-reduction, setting the C2/C1/C7 stereotriad (red arrows, Fig. 2B). This sequence would provide the entire pactamycin carbon core skeleton from which modular delivery of various functionality (Fig. 2C) could provide **1** and/or its congeners in rapid fashion.

The first challenge we faced was implementation of the Mannich reaction with an appropriately configured imine electrophile. We were encouraged by results reported by Schaus and coworkers wherein *cinchona* alkaloids were effective in catalyzing the enantioselective addition of simple 1,3-dicarbonyls to acyl imines (23, 24); however, the required asymmetric Mannich addition of α-amino-substituted dicarbonyls was heretofore unknown. Additionally, with our goal of modular construction in mind, we planned to install the unusual 1,1-dimethylurea in its native form early in our route, a tactic that was expected to obviate protection/deprotection/acylation steps that characterize all other pactamycin synthetic studies.

Pronucleophile **2** was synthesized in two steps (25) from commodity chemical acetylacetone (2.5 kg ~ $75) and subjected to adapted Mannich conditions with cinnamaldehyde-derived imine **3** (Fig. 3). An evaluation of Lewis bases led to selection of cinchonidine (**7**) as the catalyst of choice, providing Mannich product **4** in 70% isolated yield and 97:3 enantiomeric ratio (94% yield, 84:16 er before removal of the racemate by trituration). An X-ray diffraction study of a derivative (25) revealed formation of the illustrated (*R*) configuration at C2. The reader will note that this nominally corresponds to the incorrect configuration at C2, but the advancement of this stereochemical mistake was in fact critical to orchestrate downstream stereochemical outcomes and efficiently complete the synthesis (vide infra). The strategic selection of cinnamyl imine **3** as the Mannich electrophile translated to the installation all five carbons of the pactamycin core, with appropriate functional handles, in

this initial C–C bond construction. This reaction constitutes a useful advance in the synthesis of differentiated, highly functionalized 1,2-diamines by adaptation of the Schaus conditions to a new nucleophile/electrophile pair; extension to other urea/carbamate combinations can be envisaged.

The proposed desymmetrization of the Mannich adduct (**4** → **5**) is complicated by the fact that four diastereomeric mono-reduction products are possible. Lithium tri(*tert*-butoxy)aluminum hydride (LTBA) emerged as a superior reducing agent for the desymmetrization, affording hydroxyketone **5** with high diastereoselectivity (>10:1 ratio of **5**:Σ(other diastereomers)) in 72% yield. This reduction delivered the illustrated (1*R*,2*R*,7*S*)-product; therefore, the incorrect C2 isomer was parlayed into the correct C1/C7 configurations. Subsequent silyl protection of the C7 hydroxyl gave methyl ketone **6**.

Our attention then shifted to installation of the C4 side-chain and cyclization to complete the cyclopentenone core (Fig. 4). The lithium enolate of ketone **6** was treated with formaldehyde gas (generated in situ by the pyrolysis of paraformaldehyde) resulting in the single aldol addition product **8** (26, 27). Alkene ozonolysis furnished aldehyde **9** poised for intramolecular aldol condensation. Cyclization of the β-hydroxy ketone (28) was effected upon treatment with sodium methoxide to provide the five-membered pactamycin core structure (**10**) in 50% yield over two steps. Under the basic reaction conditions, the configurationally labile C2 stereocenter was inverted and only the correct C2 isomer was observed in the product enone **10**. This serendipitous event corrected our initial stereochemical error, simplifying subsequent core manipulation.

With cyclopentenone **10** in hand, three challenges remained: (i) C5 methide addition, (ii) C4 hydroxylation, and (iii) C3 aniline installation. An epoxidation/nucleophilic aniline ring-opening sequence was pursued for access to the *trans*-anilinoalcohol, inspired by a related approach by Hanessian and coworkers (16, 17). We anticipated subsequent nucleophilic methylation of the C5 ketone would complete the core functionalization. As we explored this proposed route, we discovered the importance of both the order of these steps and the protecting group identity at the C4 hydroxymethylene.

Nucleophilic epoxidation of enone **10** with basic hydrogen peroxide provided epoxy alcohol **11** with high diastereoselectivity. The sterically demanding TBDPS protecting group was imperative to ensure diastereoselective addition in the subsequent C5 methylation and to withstand the aniline epoxide-opening conditions. Installation of the silyl group provided ketone **12**, which was then treated with methyl Grignard to provide carbinol **13**, gratifyingly from the required concave facial trajectory. Nucleophile approach from the convex surface of analogous oxobicyclo[3.1.0]hexane systems is well documented (16, 17, 29) and would have provided the wrong stereochemical outcome. In the present case, we surmise that this innate preference is overridden at least in part via direction by the urea functionality, lending additional support to the strategic decision to incorporate this functionality in its native form from the outset. Epoxide **12** is the crucial branch point for analogue synthesis and has been reached in gram-quantity on a single pass. The epoxide was subjected to a $Sc(OTf)_3$-promoted nucleophilic ring-opening with 3-acetylaniline (**17**), proceeding in 66% yield with 18% recovery of the starting material to install the C3 aniline derivative. The addition of this anilino functionality in its desired, unprotected form completed functionalization of the pactamycin core (**14**).

Deprotection of both silyl ethers was accomplished upon treatment with TBAF to provide tractable tetraol **15** in 90% yield, leaving a highly reactive primary alcohol for selective acylation. A ketene-mediated acylation protocol developed by Delgado (30) and exploited by Hanessian (16, 17) proved effective in completing the sterically encumbered acylation

and providing penultimate intermediate **16**. Carboxybenzyl deprotection occurred rapidly under hydrogenolysis conditions using Pearlman's catalyst (31) in 82% yield. This deprotection completed the synthesis of pactamycin in fifteen steps and 1.9% overall yield. The route is flexible and should be amenable to the preparation of congeners since the introduction of highly-functionalized side chains in unprotected form (urea, salicylate, *meta*-acetyl aniline) has been demonstrated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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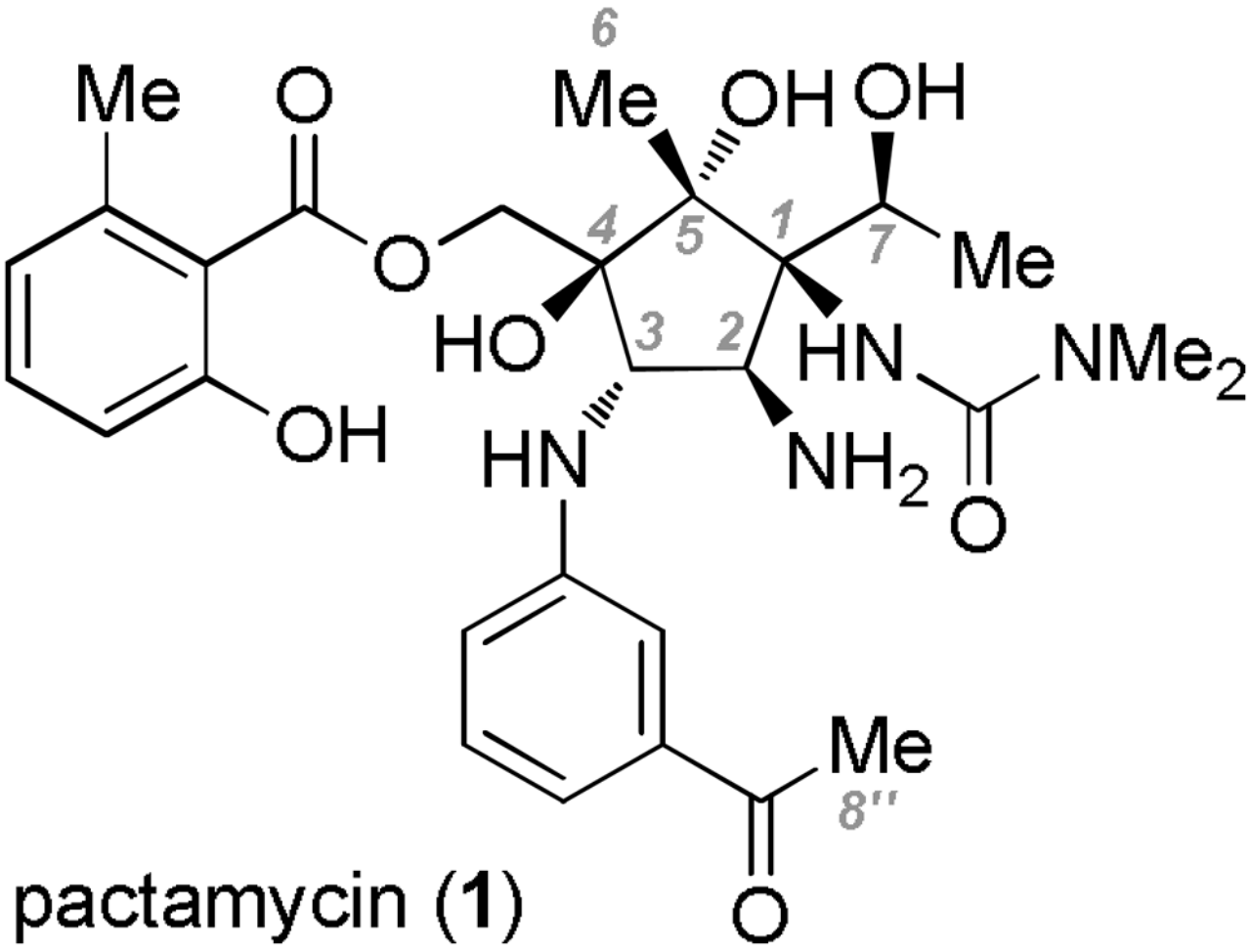

Fig. 1.
Structure of pactamycin (**1**).

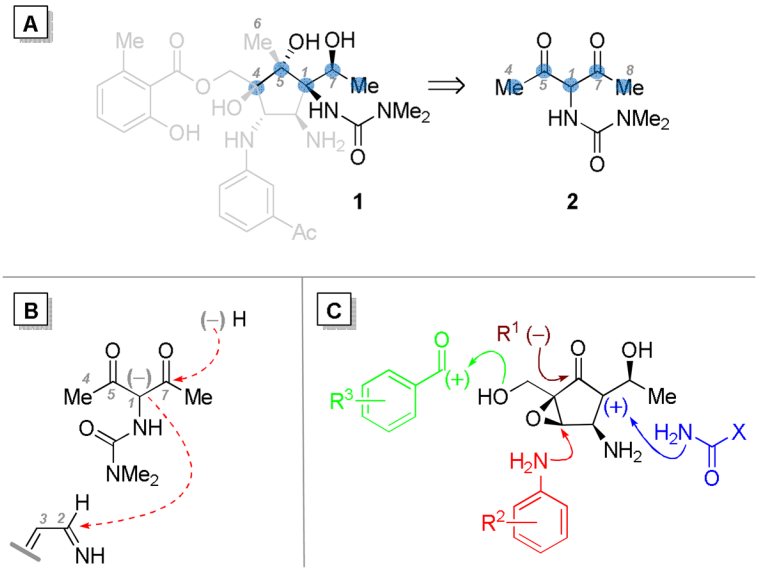

Fig. 2.

(**A**) Hidden symmetry recognition within pactamycin core. Blue dots highlight a 5-carbon chain from which to begin the synthesis. (**B**) Red arrows illustrate the pivotal Mannich addition and symmetry-breaking reduction steps. (**C**) Proposed modular construction of pactamycin. Colored components may be varied and introduced for analogue synthesis and SAR studies.

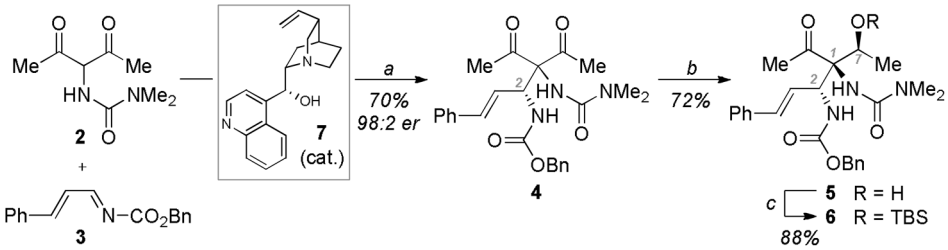

Fig. 3.

Mannich reaction and diastereoselective diketone monoreduction. This two-step sequence installs all pactamycin core carbons as well as three contiguous stereocenters. Reagents and conditions are as follows. (a) Catalyst **7** (20 mol %), dichloromethane (CH_2Cl_2), −65 °C; (b) lithium tri(*tert*-butoxy)aluminum hydride (LTBA), tetrahydrofuran (THF), −40 °C; (c) *tert*-butyldimethysilyl trifluromethanesulfonate (TBSOTf), 2,6-lutidine, CH_2Cl_2, −78 °C.

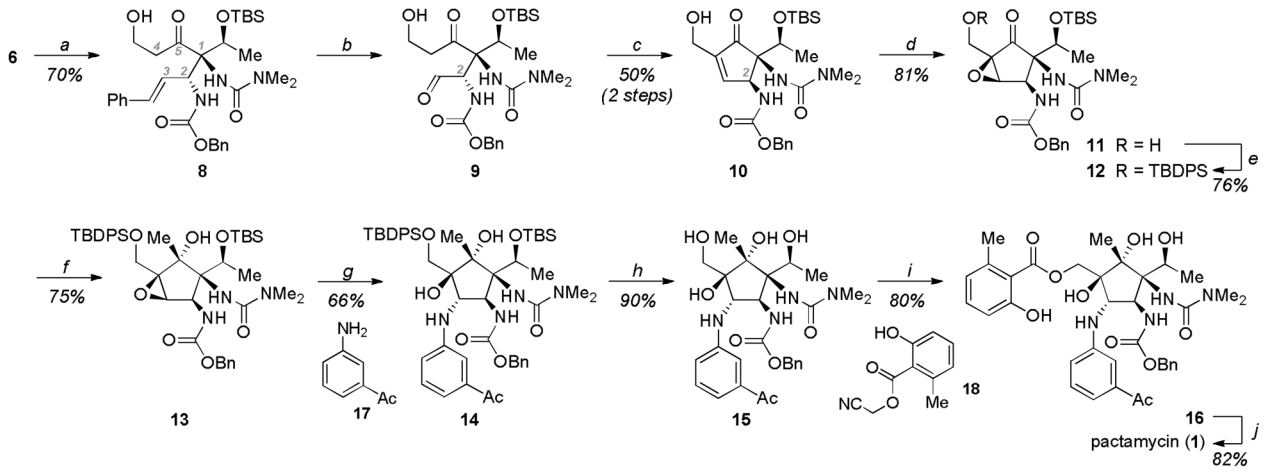

Fig. 4.

Elaboration of **6** to pactamycin (**1**) via modular incorporation of unprotected functionality. Reagents and conditions are as follows. (a) lithium diisopropylamide (LDA), formaldehyde ($CH_2O_{(g)}$), THF, −78 to −45 °C; (b) ozone (O_3), CH_2Cl_2, −78 °C, then dimethylsulfide (Me_2S), −78 °C to room temperature (rt); (c) sodium methoxide (NaOMe), THF, 0 °C; (d) hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH), 7:1 CH_2Cl_2:methanol (MeOH), 0 °C; (e) chloro(*tert*-butyl)diphenylsilane (TBDPSCl), triethylamine (NEt_3), dimethylaminopyridine (DMAP) (10 mol %), CH_2Cl_2, 0 °C to rt; (f) methylmagnesium bromide (MeMgBr), THF, 0 °C; (g) 3-acetylaniline (**17**), scandium(III) trifluoromethanesulfonate ($Sc(OTf)_3$), toluene, 60 °C; (h) tetrabutylammonium fluoride (TBAF), THF, 0 °C; (i) potassium carbonate (K_2CO_3), **18**, dimethylacetamide (DMA); (j) palladium hydroxide on carbon ($Pd(OH)_2/C$), hydrogen (H_2) (1 atm), MeOH.